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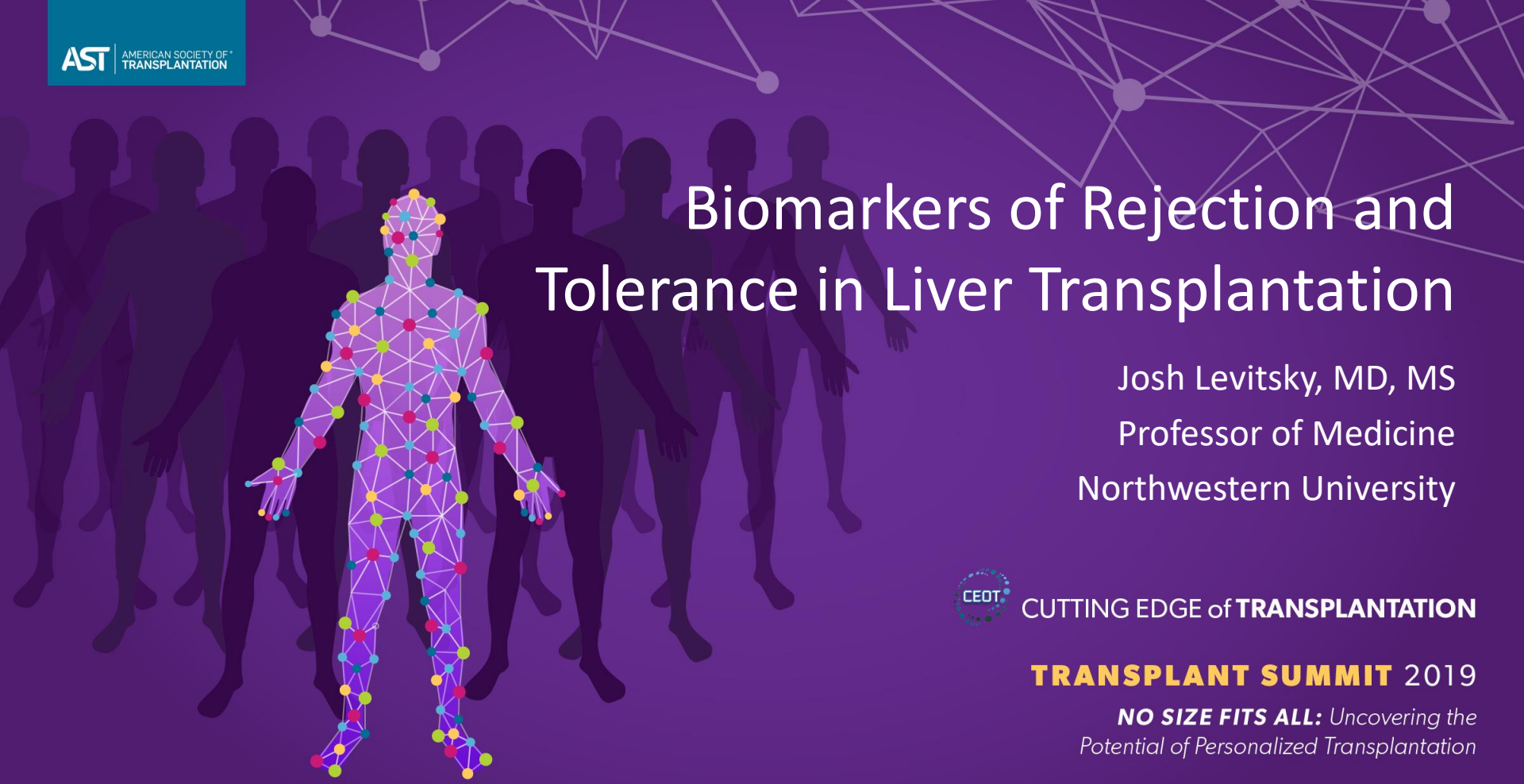

Biomarkers of Rejection and Tolerance in Liver Transplantation

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CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosures

Transplant Genomics, Inc (advisor, stockholder)
Novartis (speaker, research funding)

Learning Objectives

Review immunological issues in liver transplant recipients in the current era

Discuss which clinical situations biomarkers could advance diagnosis and management of rejection in liver transplantation

Review the current data demonstrating the use of biomarkers in liver transplant recipients

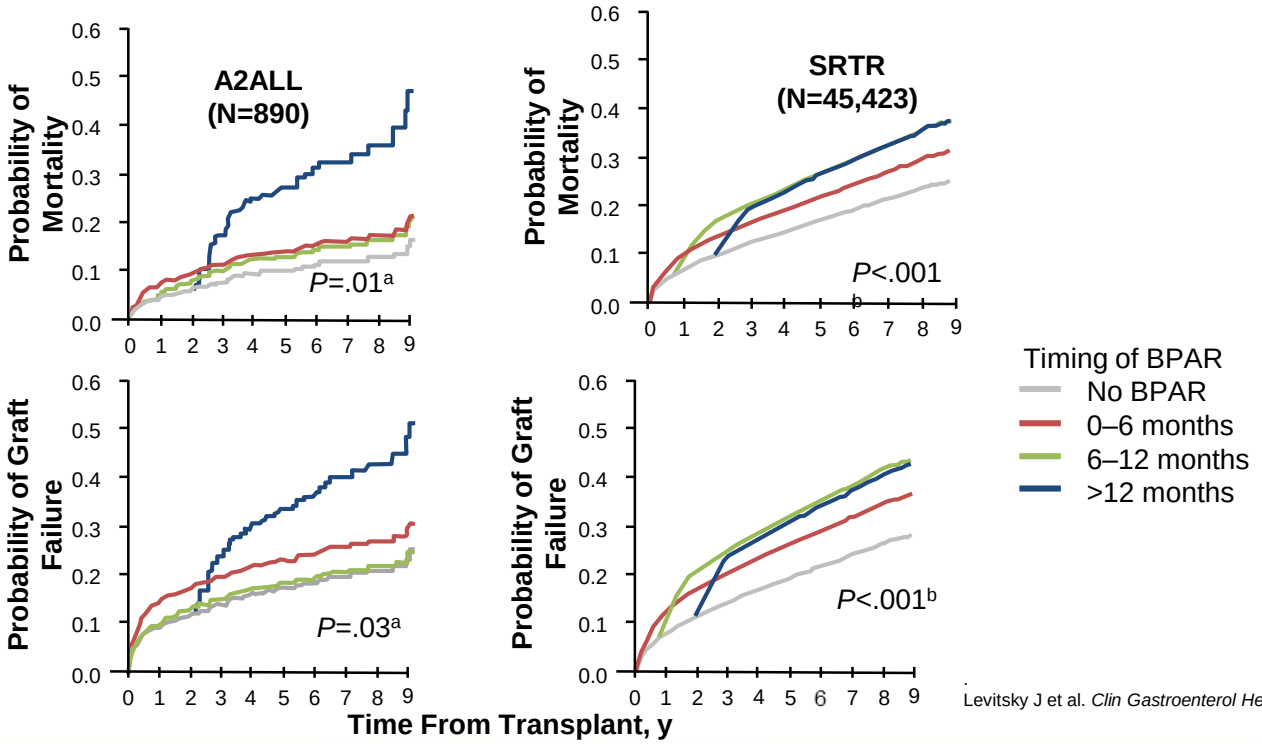
Long term complications in LTR all linked in part to IS therapy

- Malignancy – nearly half
- Cardiovascular Disease – most
- Renal dysfunction - most
- Infection – most
- Drug side effects - most
- Cost – all

- <u>Immunological graft failure: believed to be uncommon</u>

Why worry about rejection in LT recipients?

Probability of Posttransplant Mortality and Allograft Failure Over Time, by Time of First Posttransplant BPAR



Levitsky J et al. *Clin Gastroenterol Hepatol*. 2017;15(4):584-593.

Current Approach to Monitoring Level of Immunosuppression in LTR

- Clinical History
  - Age
  - History of rejection(s) vs. over-immunosuppression
  - Immune vs. Non-Immune Disease
  - Viral vs. Non-Viral Disease- no longer

- Arbitrary trough levels
  - Poor correlation with degree of immunosuppression
  - Borrowed from renal transplantation

Holy Grail

Traditional

Tolerance (Withdrawal of IS therapy with normal graft function)

Other/Alternative

Biomarker predicting rejection or other complications guiding IS modifications (augment vs. minimize)

“…..omics” for Biomarker Discovery

- Need to associate a biomarker “signature” with a “phenotype”
  - rejection, tolerance, renal disease, response to therapy

- Need to decide which compartment is most relevant (blood, urine, graft..) and cell vs. plasma vs. parenchyma

- Validation of an exploratory set is key

Practical Considerations in Transplantation

- Diagnostic biomarkers: can we make a diagnosis with a less invasive modality with equal or higher sensitivity/specificity?
  - The biomarker may be no better and more $$ than a simple serum marker (ALT)

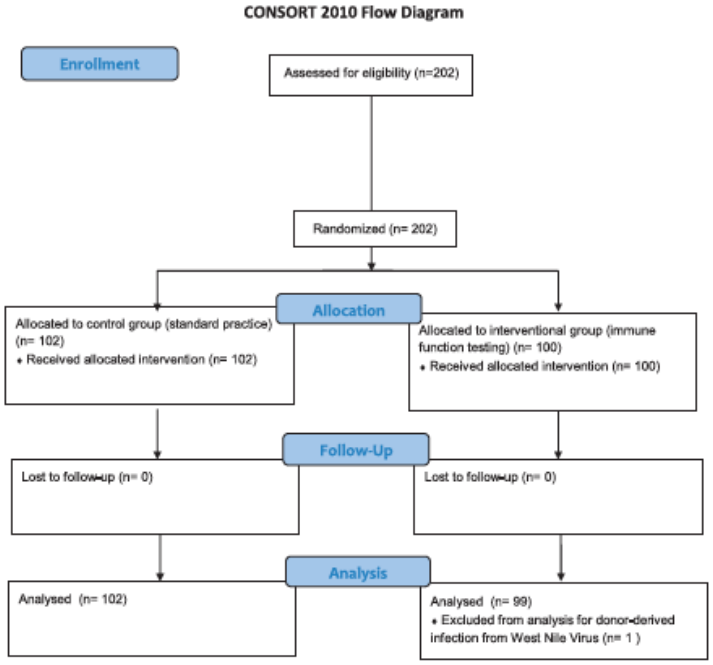
- Predictive biomarkers: can we predict biologic behavior?
  - development or progression of a condition
  - response to an intervention

- Retrospective longitudinal studies: can we use existing patients and biorepositories to inform and validate biomarker discovery?

- Prospective longitudinal studies: can we use future patients to best validate biomarker discovery?

Immunosuppression Modifications Based on an Immune Response Assay: Results of a Randomized, Controlled Trial

Matteo Ravaioli, MD,[1] Flavia Neri, MD,[2] Tiziana Lazzarotto, MD, PhD,[3] Valentina Rosa Bertuzzo, MD,[2] Paolo Di Gioia, MD, PhD,[2] Giacomo Stacchini, MD,[2] Maria Cristina Morelli, MD,[2] Giorgio Ercolani, MD, PhD,[2] Matteo Cescon, MD, PhD,[2] Angela Chiereghin, PhD,[3] Massimo Del Gaudio, MD, PhD,[2] Alessandro Cucchetti, MD, PhD,[2] and Antonio D. Pinna, MD, PhD[2]



Ravaioli et al. Transplantation 2015

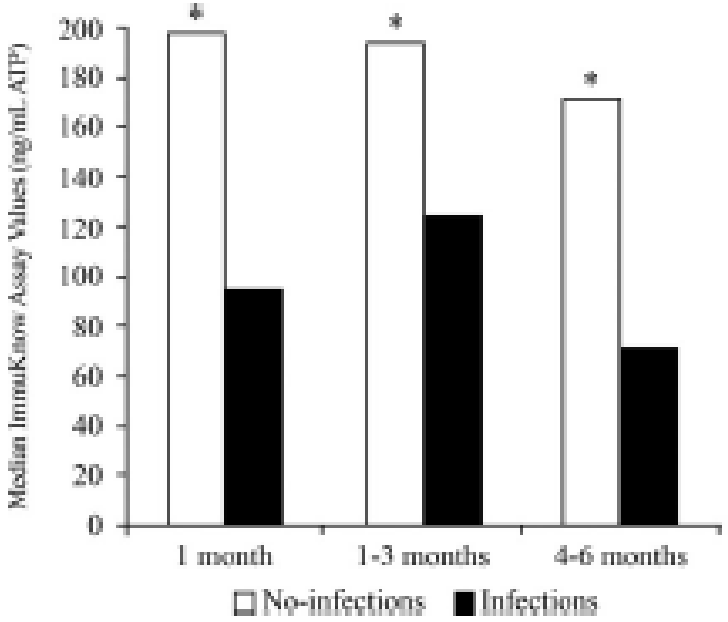
TABLE 2.

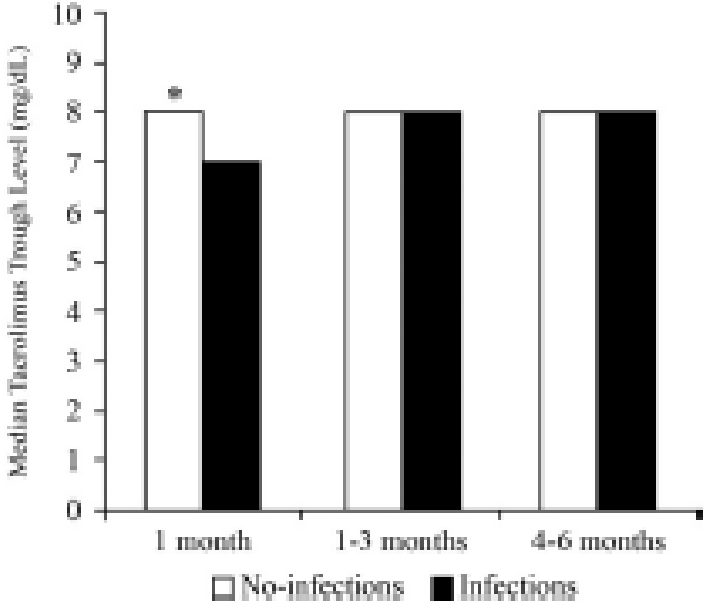
Comparison of outcomes at 12 months of follow-up

	All patients (N = 202)	Intervention group (n = 100)	Control group (n = 102)	P
Patient survival, %	170 (84.2)	89 (89.0)[a]	80 (78.4)[b]	<0.05
Event-free recipients, %	83 (58.9)	41 (41.0)	42 (41.2)	n.s.
Infectious episodes >14 d after transplantation, %	98 (48.5)	42 (42.0)	56 (54.9)	<0.05
Acute rejections, %	33 (16.3)	19 (19.0)	14 (13.7)	n.s.
Recipients with >3 infection, %	21 (10.4)	11 (11.0)	10 (9.8)	n.s.
Bacterial infections, %	77 (57.1)	32 (32.0)	47 (46.1)	<0.05
Fungal infections, %	13 (9.6)	2 (2.0)	11 (10.8)	<0.05
Viral infections, %	45 (33.3)	22 (22.0)	23 (22.5)	n.s.
	All patients, MELD >20 (N = 99)	Intervention group, MELD >20 (n = 44)	Control group, MELD >20 (n = 55)	
Patient survival, %	81 (81.8)	41 (93.2)	40 (72.7)	<0.01
Infectious episodes >14 d after transplantation, %	60 (60.6)	22 (50.0)	38 (69.1)	<0.05

[a] Cause of 11 deaths: multiple organ failure (n = 2, 18%), infections (n = 3, 27%), recurrent HCV hepatitis (n = 1, 9.1%), surgical complication (n = 2, 18%), and tumor-related causes (n = 3, 27%).
[b] Cause of 22 deaths: multiple organ failure (n = 7, 32%), infections (n = 8, 36%), recurrent HCV hepatitis (n = 2, 9.1%), technical reasons (n = 2, 9.1%), and tumor-related causes (n = 3; 14%). MELD scores were measured the day before liver transplantation.

Ravaioli et al. Transplantation 2015





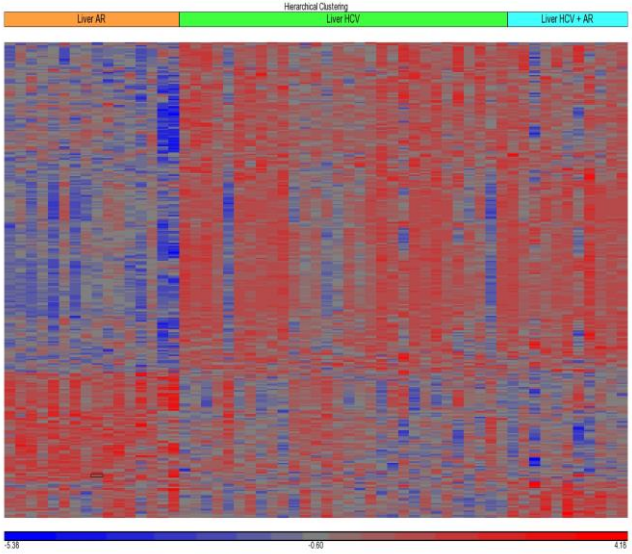
Ravaioli et al. Transplantation 2015

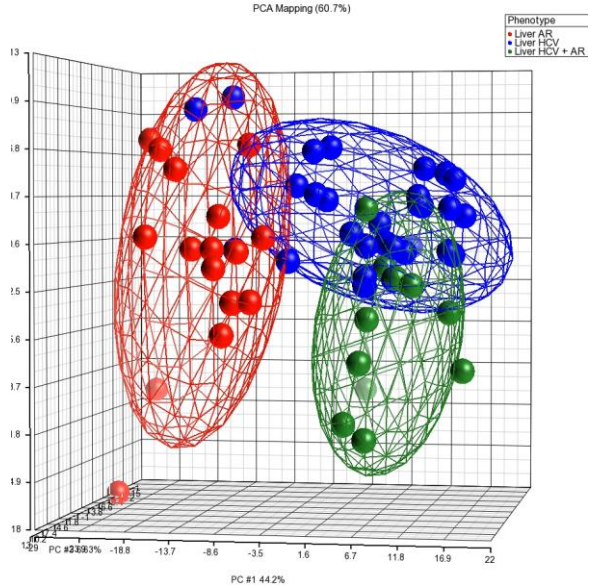
LT Rejection Biomarkers

Author	Phenotype	Source	Biomarker Result
Evans[1]	CR	Recipient DNA	HLA-DR3, TNF-2
Gomez-Mateo[2]	AR	Recipient DNA	TGFβ-1 protective
Moya-Quiles[3]	AR	Recipient DNA	Recip HLA-Cw*07 protective
Sindhi[4]	AR	Recipient DNA	rs9296068 SNP
Hanvesakul[5]	Graft function	Donor DNA	Donor HLA-C protective
Massoud[6]	AR	Recipient serum proteins	C4, C1q
Li[7]	AR	Recipient DNA and mRNA	CCL3L1 gene CNV
Karimi[8]	AR	Recipient DNA	IL-6, IFN-γ
Fan[9]	AR	Recipient PBL	Th17 cells (CD4+, IL17+)
Farid[10]	AR and injury	Recipient serum and biopsy	Hepatocyte-derived miRNA (122, 148, 194)
Joshi[11]	AR	miRNA (graft, sera)	miRNA 146, 19a, 20a, let-7e
Kamei[12]	AR	Donor and recipient DNA	GSTT1 genotype (d/r mismatch)
Shaked[13]	AR	miRNA	Can predict AR
Toby[14]	AR	Recipient PBMC proteoforms	Can predict AR and ADNR

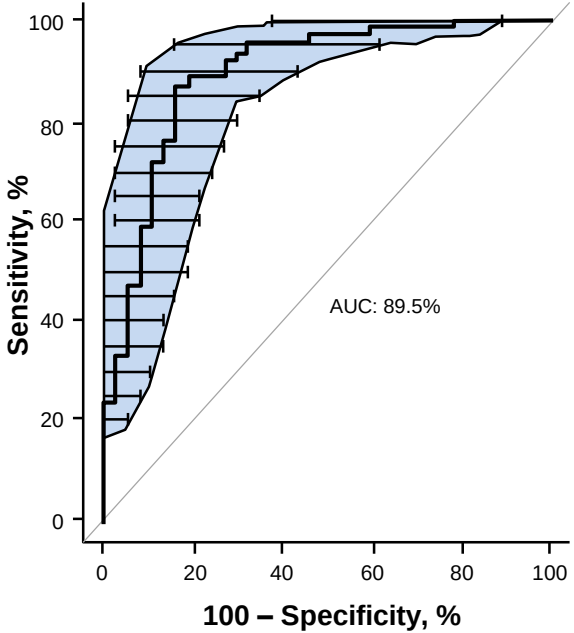
1. Evans PC. J *Hepatol*. 2001;34(5):711-715. 2. Gomez-Mateo J et al. *Transpl Immunol*. 2006;17(1):55-57. 3. Moya-Quiles MR et al. *Hum Immunol*. 2007;68(1):51-58. 4. Sindhi R et al. *Gastroenterology*. 2008;135(3):830-839. 5. Hanvesakul R et al. *Am J Transplant*. 2008;8(9):1931-1941. 6. Massoud O et al. *Liver Transpl*. 2011;17(6):723-732. 7. Li H et al. *Clin Transplant*. 2012;26(2):314-321. 8. Karimi MH et al. *Mol Biol Rep*. 2011;38(7):4437-4443. 9. Fan H et al. *Hepatobiliary Pancreat Dis Int*. 2012;11(6):606-611. 10. Farid WR et al. *Liver Transpl*. 2012;18(3):290-297. 11. Joshi D et al. *Liver Transpl*. 2013;19(4):383-394. 12. Kamei H et al. *Transpl Immunol*. 2013;28(1):14-17. 13. Shaked A et al. *Hepatology*. 2017;65(1):269-280. 14. Toby TK et al. *Am J Transplant*. doi: 10.1111/ajt.14359. Accessed May 25, 2017.

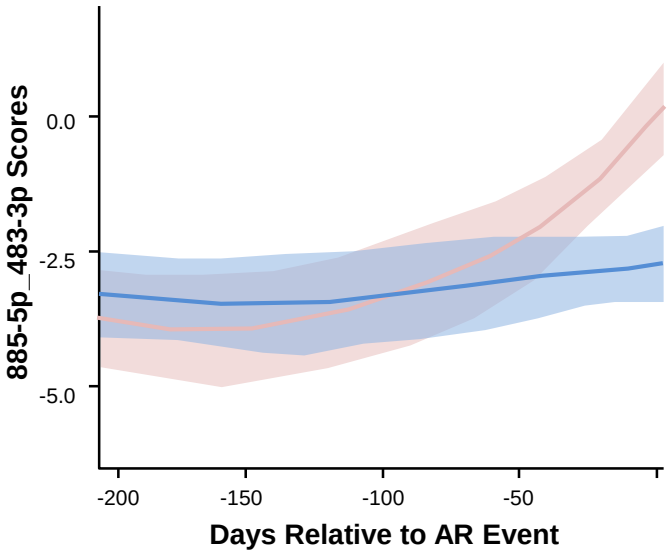
Biopsy mRNA: AR vs. HCV-R vs. Mixed AR/HCV-R





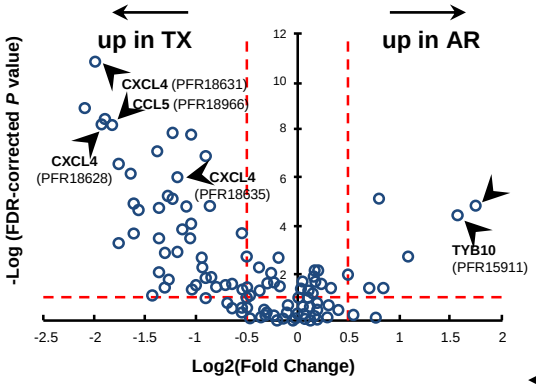
An Ectopically Expressed Serum miRNA Signature Prognostic and Diagnostic of Liver Allograft Rejection

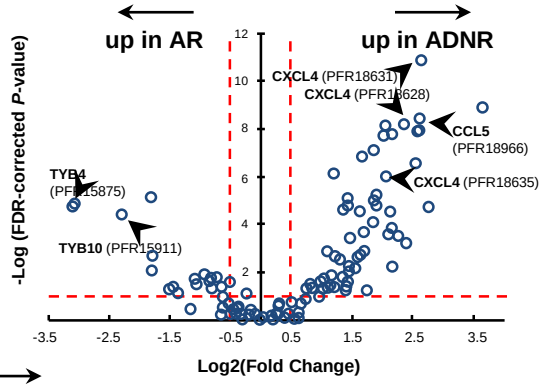


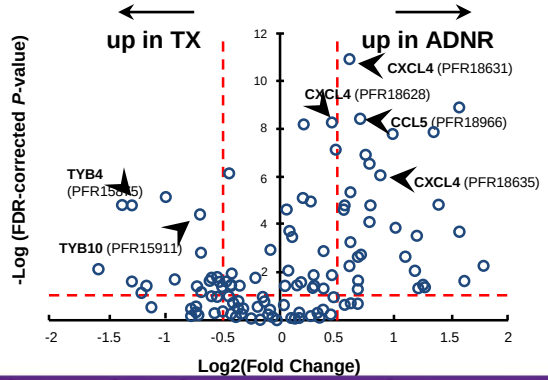


Shaked A et al. *Hepatology*. 2017;65(1):269-280.

Proteoforms of Acute Rejection in LT







Toby TK et al. *Am J Transplant*. 2017

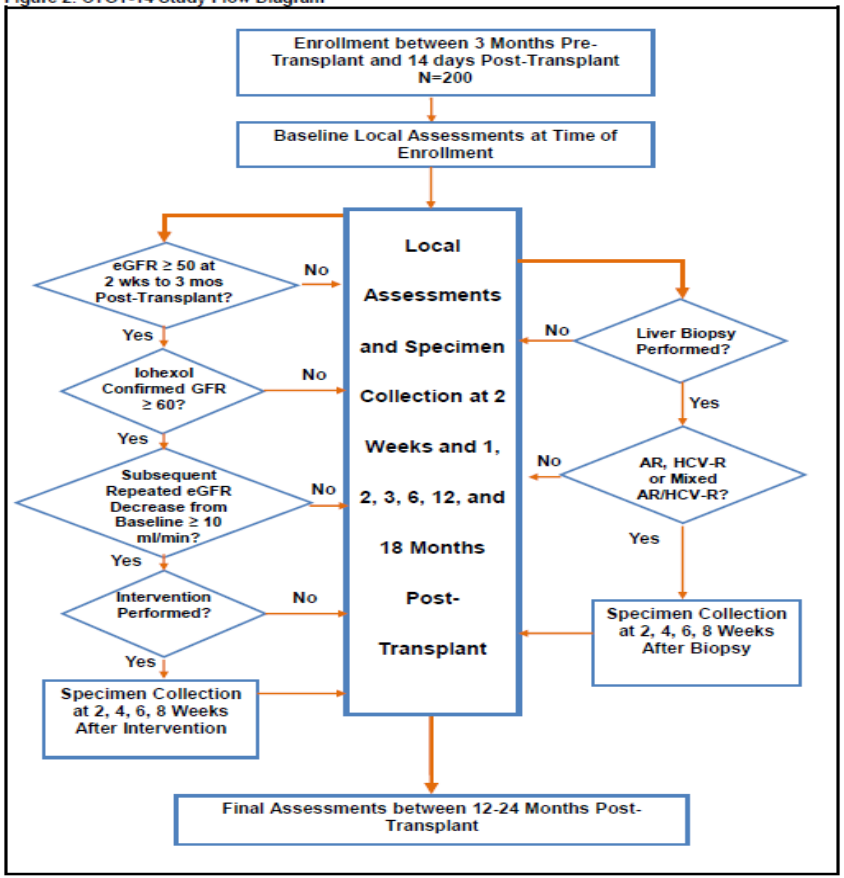
CTOT-14:

Prospective sample collection from the time of LT in 202 recipients (7 centers)

<u>Main objective:</u>

Diagnostic and predictive genomic signatures of AR and CKD in LTR

Figure 2. CTOT-14 Study Flow Diagram



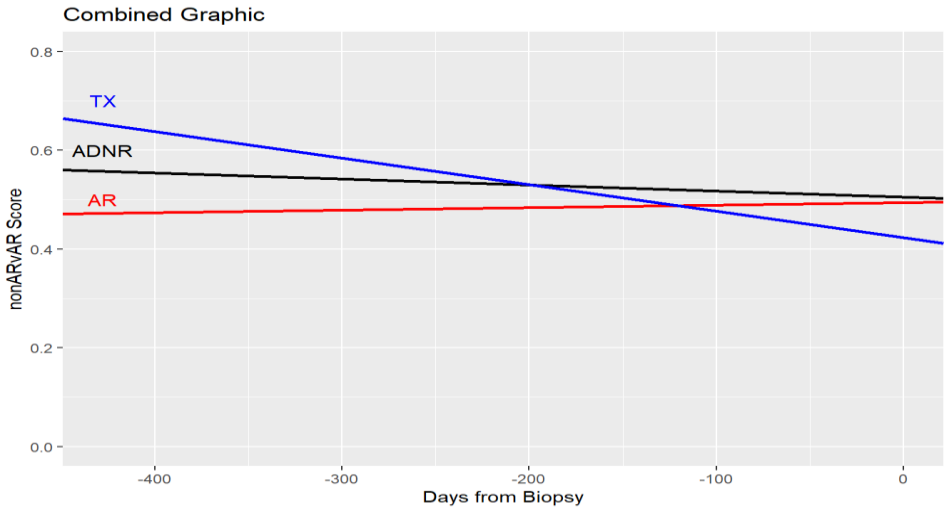
CTOT-14 Primary Objective: AR Diagnosis (AR vs. TX)

	AUC	Accuracy	Sensitivity	Specificity	PPV	NPV
NU Discovery Cohort	0.92	0.80	0.76	0.84	0.58*	0.93*
CTOT14 Validation Cohort	0.72	0.81	0.50	0.90	0.58	0.87

*Prevalence-adjusted PPV and NPV

PREDICTING AR vs. TX (quiescence) in LTR

Pre-AR, Pre-ADNR, Pre-TX have different trajectories over time (p<0.001)

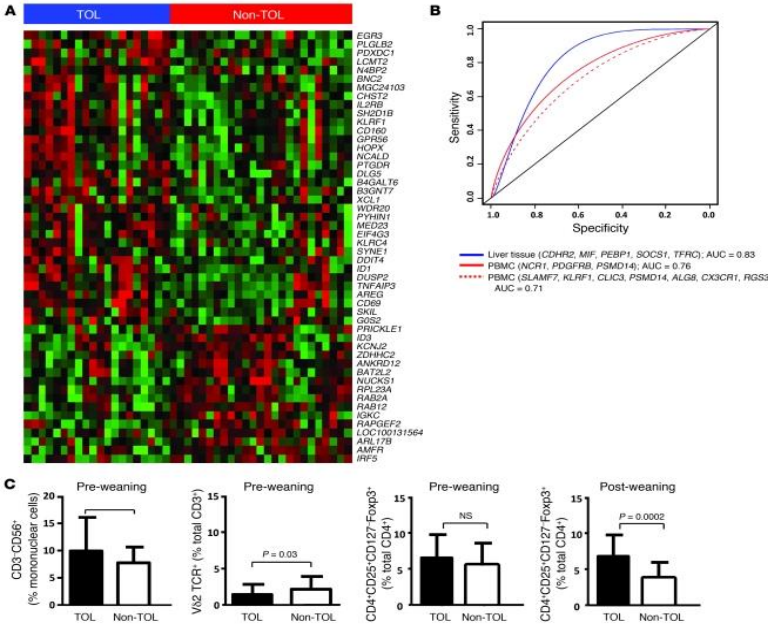


High NPV pre-AR, leading to confidence in detecting immune quiescence in serial monitoring

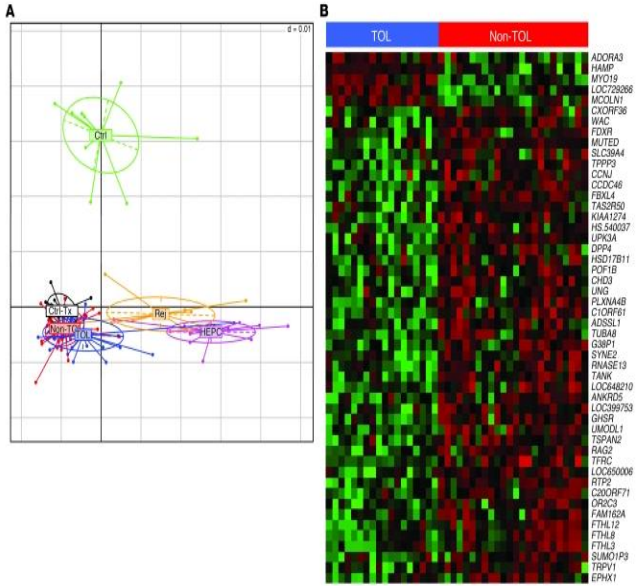
	Sensitivity	Specificity	PPV	NPV
Pre-AR vs. Pre-TX	0.26	0.75	0.17	0.84
Pre-AR vs. Pre-nonAR	0.26	0.71	0.13	0.85

Blood vs. Graft Transcripts Pre-Withdrawal

PBMC

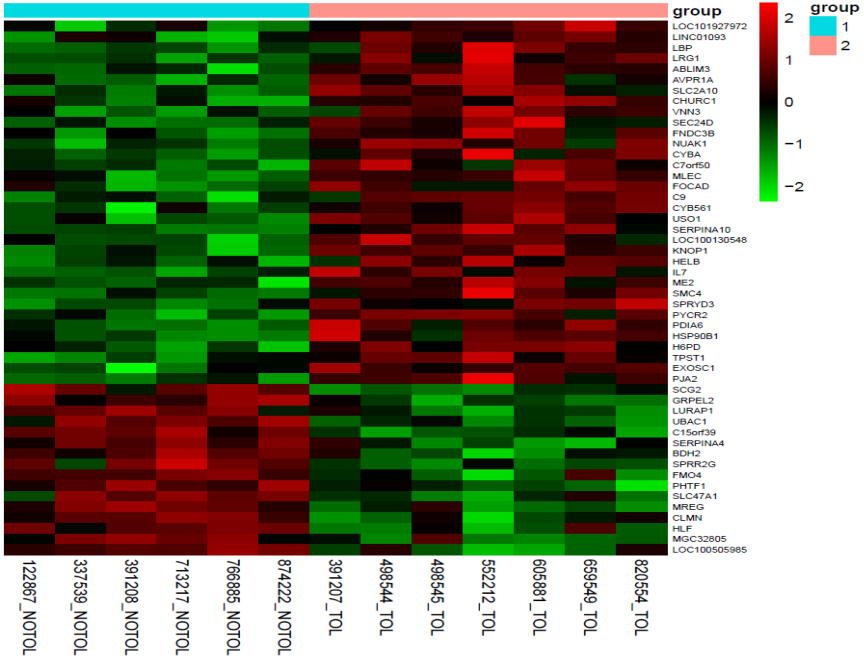


Allograft



Bohne et al. JCI 2012

Biopsy Gene Expression Profiling – mTOR-I withdrawal (TOL vs. non-TOL)



93 probes in biopsy

- distinguish TOL vs. non-TOL at baseline
- Minimal similarity between biopsy and blood genes

* A previously identified biopsy gene signature accurately predicted TOL in 12/14 (85.7%)

* Bohne et al. J Clin Invest. 2012 Jan;122(1): 368-82

Levitsky et al. AASLD 2017

Future Directions

- Non-invasive biomarkers of rejection in Liver Transplant
  - Few clinical applications as of yet
  - Most are diagnostic at time of AR

- High need for predictive biomarkers to advance clinical management
  - Prior to acute rejection
  - Prior to IS minimization or full withdrawal
  - Select patients for specific monitoring and interventions, such as augmentation or minimization of immunosuppression

- Need randomized controlled trials testing biomarkers vs. standard of care in managing immunosuppression (optimization) to improve outcomes

THANKS